Novel diagnosis and progression microRNA signatures in melanoma

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Keywords

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ABSTRACT

Melanoma, a highly aggressive skin cancer with a high mortality rate, has seen an increase in its incidence over the years. While there is a differential pattern based on gender, there is a lack of knowledge regarding efficient biomarkers that can aid in the early detection and management of the disease in patients.

This study conducted a systematic review of melanoma studies following PRISMA guidelines. Subsequently, exploratory analysis and data normalization were carried out. Individual analyses of each study were performed, followed by a meta-analysis that integrated all the results, taking patient information into account.

The findings have significantly contributed to a better understanding of the molecular mechanisms and developmental processes involved in melanoma. This, in turn, offers the potential for the development of novel resources for the precise detection and treatment of melanoma within the context of precision medicine.

INTRODUCTION

Cutaneous melanoma is an aggressive skin cancer characterized by a high metastatic potential and lethality. In fact, melanoma is one of the most aggressive tumors, and although it constitutes only about 5% of skin cancers, producing the death of 80% of patients. Moreover, its incidence, recurrence, and mortality rate continue to grow worldwide, especially in fair-skinned populations (Sandru et al,2014; Atkins et al, 2021).

The melanoma affects the pigment-producing cells, located in the skin surface (Atkins et al, 2021), being the most common symptoms changes in size, shape, or color (O'Shea et al,217). Additionally, the survival of patients with melanoma depends on several factors, but on Breslow's primary lesion thickness and an early diagnosis are the most critical (Naik et al.,2023).

An early diagnosis is considered when during the stage of radial development, the time in which the neoplastic cells are confined to the epidermis is detected. Population-oriented campaigns focused on melanoma risk and clinical warning signs can improve access to specialists for prompt diagnosis and treatment (Kaiser et al, 2014; Goyal et al. 2020). However, the melanoma presents late onset of symptoms, making difficult an early detection of the disease. In addition, the detection remains complicated since, in terms of molecular and clinical features, melanomas are heterogeneous, but dynamic changes in the tumor environment can also lead to immune surveillance escape. Thereby, understanding the heterogeneity is critical for the patient treatment (Fares et al., 2020; Kirstein et al.,2016; Nirschl et al. 2017; Neagu et al. 2010).

The clinical diagnosis is based on morphological criteria analysis, performed by visual examination aided by lesion dermoscopy. This procedure can be subjective and, in addition to visual examination, an excision is performed when a lesion is suspected of having melanoma (Kaiser et al, 2014; Goyal et al. 2020). Indeed, when the cancer is diagnosed in early stages, surgical removal of the tumor is the appropriate option to improve patient survival. Nevertheless, the metastatic potential of melanoma compromises the efficacy of surgery to solve the problem in more advanced stages of the disease (Erdei et al, 2010).

Currently, the development of new non-invasive technologies such as artificial intelligence for diagnosis skin cancer is emerging to support the rest of the clinical parameters (Kaiser et al, 2014; Goyal et al. 2020). In this sense, the detection of changes in the miRNA profile in tumor tissue may serve as a tool for identification of novel molecular markers (Komina et al 2016) and predict disease diagnosis or progression. However, there are still gaps in research such as, among others, the clinical utility and prognostic significance of some biomarkers and molecular tests, the value of ancillary molecular tests in comparison with well-established clinicopathologic predictors of outcome (Swetter et al., 2019), and the he lack of effective biomarkers that allow the early and accurate detection of this disease or the prediction of the evolution of the disease in

a patient once it has been detected. All these factors make the diagnosis of melanoma problematic at present.

Regarding molecular factors of melanoma, microRNA (miRNA) expression patterns show dysregulation during progression of the disease (Mueller et al.,2009; Jayawardana et al., 2016). The miRNAs are short non-coding RNAs regulating gene expression in many biological processes, including proliferation, apoptosis, and differentiation(Ambros et al, 2021; Bartel et al.,2004; He et al.,2004). However, according to functional and expression studies in cell lines, altered miRNA expression is critical to disease development (Mueller et al.,2009,Gaur et al.,2007).

MiRNAs are essential regulators of gene expression at the post-transcriptional level. Their expression is altered in cancer tissues, and evaluation of these alterations is considered a promising tool used to diagnose and identify prognostic markers (Komina et al 2016). Other studies (Caramuta et al., 2010), showed several miRNA were differentially underexpressed in cases with a BRAF mutation (miR-193a, miR-338, and miR-565) in melanoma. In addition, a low expression of miR-191 and high expression of miR-193b were associated with poor melanoma-specific survival.

MiR-4286 was shown to be up-regulated in different types of malignancies such as pancreatic cancer (Frampton et al., 2014), or esophageal adenocarcinoma (Drahos et al., 2016). Furthermore, other studies included miR-4286 in a list of significantly up-regulated microRNAs in melanoma whose role in melanoma genesis has not been studied (Sand et al., 2013). In addition, Komina et al., (2016), showed that miR-4286 contributes to the regulation of melanoma cell proliferation and apoptosis by triggering several pathways, suggesting that it exerts a pleiotropic effect. In addition, some biological functions (fatty acids biosynthesis, elongation, degradation and metabolism) were altered in melanoma compared to nevi samples. Nevertheless,

a specific microRNA has not yet been shown as an adequate marker to diagnose and predict the prognosis of patients with this disease (Komina et al 2016).

On the other hand, a mutated form *CDKN2A* gene in chromosome 9 has been involved with a high risk for melanoma development. Moreover, under normal conditions, this gene plays an important role in tumor cells growth but if the gene mutates, the action of the tumor suppressor is lost and cancer cells develop uncontrollably (Burns et al. 2019).

A crucial tool for advancing the identification of new biomarkers in melanoma skin cancer is Bioinformatics. This science plays a fundamental role since the combination of different computational tools to process large volumes of biological data allows the detection of biomarkers such as microRNAs (miRNAs), the development of new genetic treatments and even a better use of existing scientific knowledge.

Therefore, considering all the factors mentioned above, the general hypothesis of this work is that an integrative computational approach of all published studies in melanoma with miRNAs markers can contribute to a better understanding of the molecular mechanisms in melanoma, the regulatory processes that occur, and generate new strategies and resources for an early detection and treatment. Consequently, the overall objective of this work was the detection of the different differential molecular mechanisms behind melanoma, such as differential expression of miRNAs. Sometimes, we know that these differences exist through clinical and epidemiological indicators, but we do not know exactly what they are due to. This information would allow us to more precise diagnosis, disease progression, and treatment selection for each group of patients.

RESULTS

Systematic review

The research process resulted in the identification of 57 studies from the GEO and ArrayExpress databases. After conducting a thorough process of deduplication and the inclusion of multi-part studies, a final corpus of 54 unique studies was obtained. After the subsequent stringent filtering criteria applied, we excluded those which were not primarily oriented to melanoma, studies utilizing cell line-based, and those featuring an insufficient number of samples, culminating in a final selection of 9 studies to be included in this work.

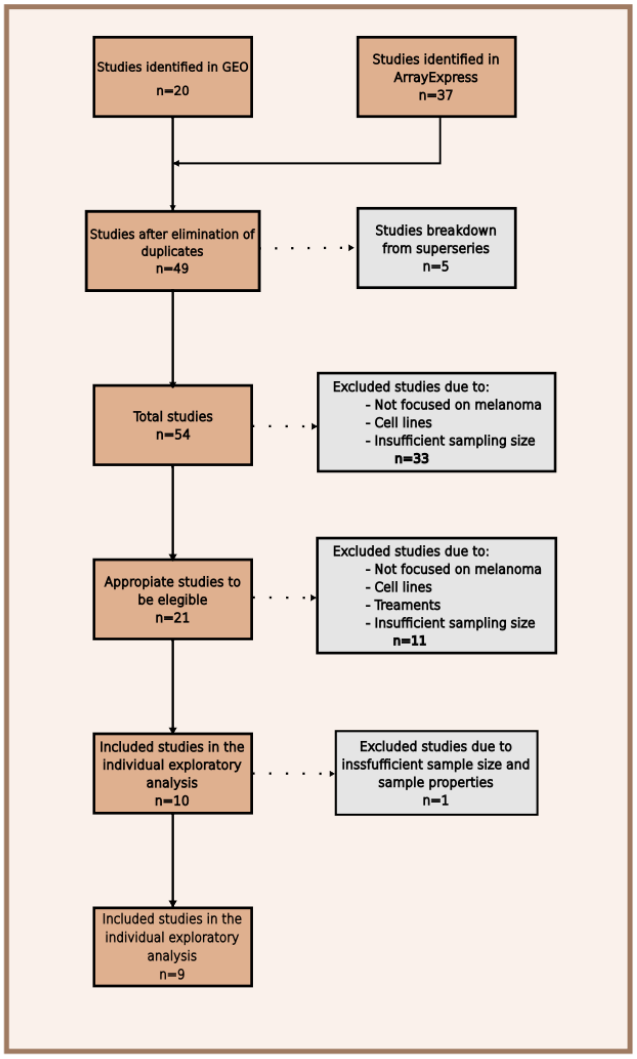

Figure 1. PRISMA diagram of the systematic review.

Data description

Mainly tissue samples were focused on primary cutaneous melanoma (PM), benign nevi (BN), metastatic cutaneous or lymphatic melanoma. Number of samples by tissue and study is detailed in Table 1.

Table 1. Description of selected studies. The characteristics of the studies are shown as available in its respective database.

Accession	Database/Platform	Tissue (nº samples)	Reference
EMTAB4915	ArrayExpress/-	➔ Primary melanoma (16) ➔ Benign nevi (3)	Ruksha et al., (2017)
GSE18509	GEO/GPL9081	➔ Metastatic melanoma (8) ➔ Benign nevi(8)	Chen et al. (2010)
GSE54492	GEO/GPL10262	➔ Primary melanoma(8) ➔ Lymph node metastases (9) ➔ Distant cutaneous metastases(8)	Bhattacharya et al., (2015)
GSE34460	GEO/GPL10262	➔ Primary melanoma(9) ➔ Benign nevi (8) ➔ Cutaneous melanoma metastases (4)	Sand et al. (2013)
GSE59334	GEO/GPL15018	➔ Melanoma lymph node(74)	Jayawardana et al. (2015)
GSE62370	GEO/ GPL19295	➔ Primary melanoma(92) ➔ Benign nevi (9)	Hanniford et al.(2015)
GSE62371	GEO/GPL19296	➔ Primary melanoma (119)	Hanniford et al.(2015a, 2015b)
GSE211090	GEO/ GPL32482	➔ Primary melanoma (35)	DiVincenzo et al. (2023)
GSE211092	GEO/GPL32482	➔ Primary melanoma (12)	DiVincenzo et al. (2023)

Individual Analysis

We carried out exploratory and processing steps on the datasets to ensure their comparability in subsequent analyses. We applied $\log_2$ transformation to studies GSE when necessary for

normalization. In the diagnosis scenario, three and four studies were included in PM vs BN and MM+PM vs BN, respectively. Regarding the prognosis scenario, four studies were included in UM vs NUM, meanwhile three studies were included in the survival approach.

Scenarios Approach

In the diagnostic context, three studies were incorporated into the comparison between primary cutaneous melanoma (PM) and benign nevi (BN), while four studies were included in the comparison between metastatic melanoma (MM) and the combined group of primary and metastatic cutaneous melanoma versus benign nevi (MM+PM vs BN).

Concerning the progression scenario, four studies were included in the comparison of ulcerated melanoma (UM) versus non-ulcerated melanoma (NUM), while three studies were integrated into the survival analysis.

Differential Expression Analysis

The number of significantly differentially-expressed miRNAs by study and contrast can be observed in Figure 2 and Table S1. Study GSE54492 was not included in any group of contrast due to its samples being totally different to others. However, its differential expression analysis revealed 109 miRNA downregulated in primary melanoma vs metastatic melanoma. On the other hand, no study included in the survival approach showed significant miRNAs differential expression. Regarding the ulceration contrast only GSE62370 and GSE62371, revealed 2 and 4 miRNA up-regulated, respectively.

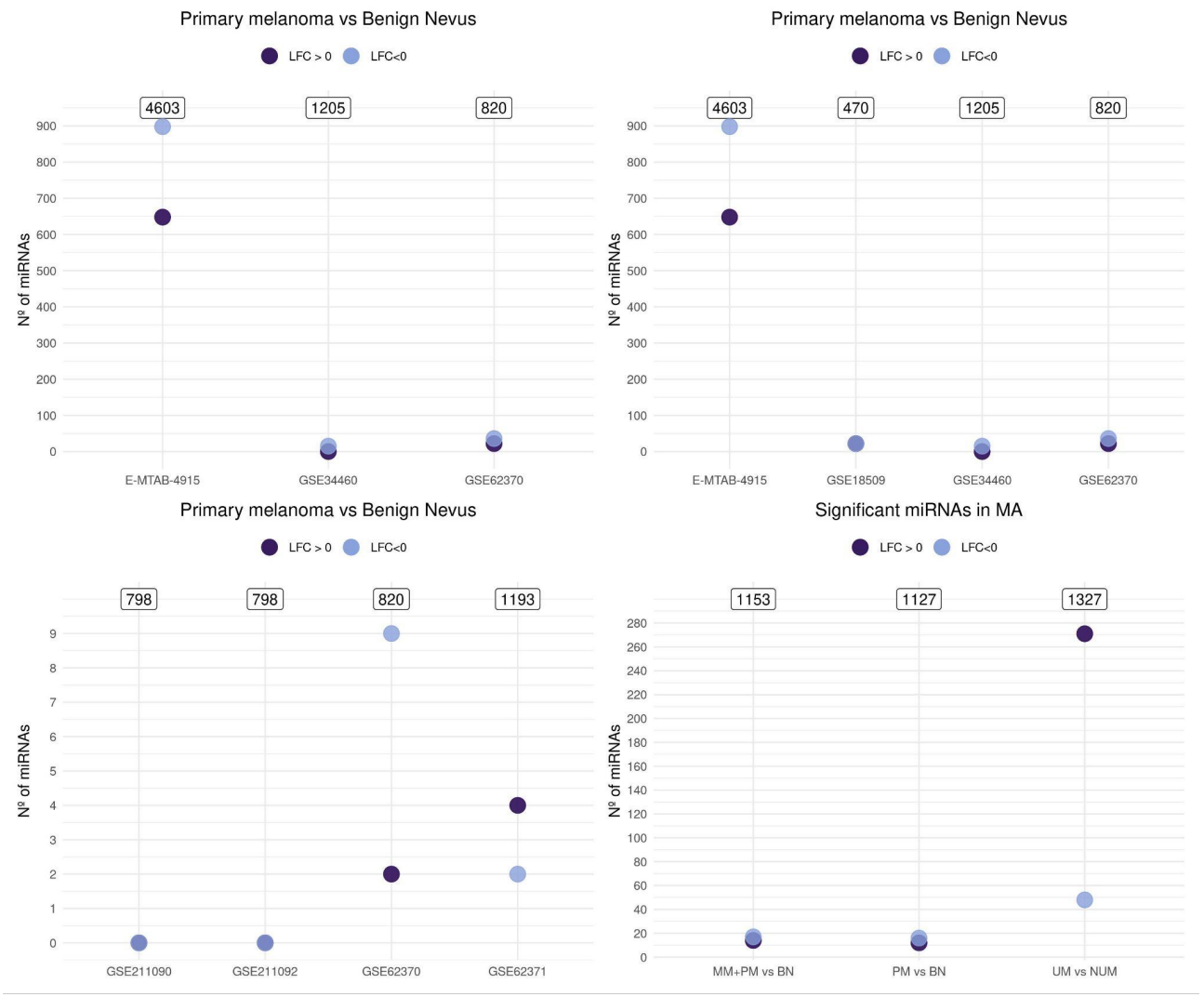

Figure 2. Number of significantly differentially-expressed miRNAs by study and contrast in MA. Description of significant miRNAs by comparisons: Primary melanoma (PM) vs Benign nevus (BN), Primary melanoma and metastatic melanoma(PM+MM) vs Benign nevus (BN), and ulcerated melanoma (UM) vs non-ulcerated melanoma (NUM). Labels showed total miRNAs analyzed. LFC: log-FoldChange.

Description of significant miRNAs in the individual differential expression analysis can be observed in Table 2.

Table 2. Description of significant miRNAs by study. Significant miRNAs are grouped according to the sign of their Log fold-change (LFC).

Study	LFC	significant miRNAs
E-MTAB-4915	**LFC > 0**	hsa-mir-4662b; hsa-miR-580-3p; hsa-mir-3682; hsa-miR-510-3p; hsa-miR-3179; hsa-miR-3915; hsa-miR-4782-5p; hsa-miR-5591-5p; hsa-mir-4517; hsa-mir-557; hsa-mir-6073; hsa-miR-105-3p; hsa-miR-6853-5p; hsa-miR-3164; hsa-miR-3611; hsa-miR-1293; hsa-mir-664b; hsa-miR-6878-5p; hsa-miR-6130; hsa-mir-8063; hsa-mir-320b-1; hsa-miR-3925-5p; hsa-mir-3945; hsa-mir-371b; hsa-miR-627-5p; hsa-miR-4764-5p; hsa-mir-1183; hsa-mir-520f; hsa-mir-4748; hsa-miR-3202; hsa-mir-184; hsa-miR-32-3p; hsa-miR-4419b; hsa-mir-3150a; hsa-mir-4300; hsa-miR-4753-5p; hsa-miR-4675; hsa-miR-4278; hsa-mir-200b; hsa-miR-1321; hsa-miR-5008-5p; hsa-miR-4737; hsa-miR-7856-5p; hsa-mir-4518; hsa-miR-4458; hsa-mir-21; hsa-mir-3139; hsa-miR-5186; hsa-miR-6857-5p; hsa-mir-494; hsa-miR-6864-5p; hsa-miR-3936; hsa-miR-4700-5p; hsa-mir-4701; hsa-mir-6855; hsa-mir-6892; hsa-mir-4747; hsa-mir-363; hsa-miR-6788-5p; hsa-miR-4450; hsa-miR-3163; hsa-mir-933; hsa-miR-5191; hsa-miR-4499; hsa-miR-4468; hsa-miR-6770-5p; hsa-mir-4690; hsa-mir-4313; hsa-miR-4506; hsa-miR-6837-5p; hsa-miR-4715-5p; hsa-miR-659-3p; hsa-mir-4673; hsa-miR-5187-5p; hsa-miR-3137; hsa-miR-4647; hsa-miR-4451; hsa-mir-3609; hsa-miR-4672; hsa-miR-5194; hsa-miR-299-3p; hsa-miR-4638-5p; hsa-miR-7843-5p; hsa-miR-4769-5p; hsa-miR-3173-3p; hsa-miR-3186-3p; hsa-miR-6509-5p; hsa-miR-3198; hsa-mir-6846; hsa-miR-6822-5p; hsa-miR-920; hsa-miR-3646; hsa-miR-6883-5p; hsa-miR-6515-5p; hsa-miR-1273e; hsa-miR-6747-5p; hsa-miR-4472; hsa-miR-1270; hsa-miR-4481; hsa-miR-760; hsa-miR-4747-5p; hsa-miR-3132; hsa-miR-4454; hsa-miR-4436b-3p; hsa-miR-3622a-5p; hsa-miR-6865-5p; hsa-miR-6890-5p; hsa-mir-6723; hsa-miR-4533; hsa-miR-5698; hsa-mir-320a; hsa-miR-4773; hsa-miR-6884-5p; hsa-miR-6758-5p; hsa-miR-4653-3p; hsa-miR-4259; hsa-miR-6804-5p; hsa-miR-2467-3p; hsa-miR-6810-5p; hsa-miR-650; hsa-miR-3138; hsa-miR-648; hsa-miR-3190-3p; hsa-miR-4644; hsa-miR-4535; hsa-miR-6892-5p; hsa-miR-4476; hsa-miR-652-5p; hsa-miR-557; hsa-miR-6835-5p; hsa-mir-6876; hsa-miR-564; hsa-miR-3918; hsa-mir-500a; hsa-miR-6867-5p; hsa-miR-6730-5p; hsa-miR-4716-3p; hsa-miR-6881-5p; hsa-miR-3610; hsa-miR-4317; hsa-miR-371a-5p; hsa-miR-425-3p; hsa-mir-4284; hsa-mir-4716; hsa-miR-2276-3p; hsa-miR-4783-3p; hsa-miR-4461; hsa-miR-5088-5p; hsa-miR-135b-3p; hsa-miR-6871-5p; hsa-miR-3191-3p;hsa-miR-604; hsa-miR-4748; hsa-miR-575; hsa-miR-4673; hsa-mir-424; hsa-miR-6754-5p; hsa-miR-6744-5p; hsa-miR-6751-5p; hsa-miR-6855-5p; hsa-miR-1183; hsa-miR-363-5p; hsa-mir-4507; hsa-miR-4441; hsa-mir-611; hsa-miR-6847-5p; hsa-mir-6825; hsa-miR-4478; hsa-miR-5703; hsa-miR-3200-5p; hsa-mir-6090; hsa-miR-3151-5p; hsa-mir-3607; hsa-miR-4738-3p; hsa-mir-663a; hsa-miR-298; hsa-miR-6511a-5p; hsa-miR-4489; hsa-miR-711; hsa-miR-365a-5p; hsa-mir-941-1; hsa-mir-941-2; hsa-mir-941-3; hsa-mir-941-4; hsa-miR-4496; hsa-miR-1910-3p; hsa-miR-6748-5p; hsa-miR-5195-3p; hsa-miR-658; hsa-miR-424-3p; hsa-miR-6784-5p; hsa-miR-4455; hsa-miR-3121-3p; hsa-miR-1909-3p; hsa-miR-4713-3p; hsa-miR-4709-3p; hsa-miR-3124-5p; hsa-miR-4271; hsa-miR-30c-1-3p; hsa-miR-2115-3p; hsa-miR-1303; hsa-miR-3934-5p; hsa-miR-6717-5p; hsa-miR-4657; hsa-mir-4634; hsa-miR-4750-5p; hsa-miR-3926; hsa-mir-6858; hsa-mir-423; hsa-miR-6772-5p; hsa-miR-6721-5p; hsa-miR-5572; hsa-mir-1248; hsa-miR-10b-3p; hsa-mir-486-2; hsa-miR-34c-5p; hsa-miR-4684-3p; hsa-miR-8052; hsa-miR-6781-5p; hsa-miR-4731-5p; hsa-miR-5010-5p; hsa-miR-550b-2-5p; hsa-miR-1972; hsa-mir-3656; hsa-miR-4442; hsa-miR-1226-5p; hsa-miR-30c-2-3p; hsa-mir-664a; hsa-miR-769-5p; hsa-miR-6740-5p; hsa-mir-3155b; hsa-mir-4430; hsa-miR-8071; hsa-miR-1292-5p; hsa-miR-3682-3p; hsa-miR-3622b-5p; hsa-miR-6076; hsa-miR-6792-5p; hsa-mir-4419a; hsa-miR-6726-5p; hsa-miR-1238-5p; hsa-miR-8055; hsa-miR-6796-5p; hsa-miR-6886-5p; hsa-miR-1273f; hsa-miR-6815-5p; hsa-mir-1233-1; hsa-mir-1233-2; hsa-miR-324-3p; hsa-miR-5093; hsa-miR-4419a; hsa-miR-8060; hsa-miR-887-3p; hsa-miR-4776-5p; hsa-miR-4525; hsa-miR-1193; hsa-miR-4510; hsa-miR-501-3p; hsa-miR-612; hsa-miR-6075; hsa-miR-4722-5p; hsa-miR-4322; hsa-mir-6819; 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hsa-miR-3939; hsa-miR-2115-5p; hsa-mir-6777; hsa-mir-4329; hsa-mir-6857; hsa-mir-1281; hsa-miR-5690; hsa-miR-6812-3p; hsa-miR-3677-3p; hsa-mir-2392; hsa-mir-6799;hsa-mir-657; hsa-miR-6779-3p; hsa-mir-7-2; hsa-miR-4473; hsa-mir-4641; hsa-miR-6856-3p; hsa-mir-4516; hsa-mir-320b-2; hsa-mir-6820; hsa-miR-5588-3p; hsa-miR-7974; hsa-mir-6510; hsa-miR-329-3p; hsa-miR-6513-3p; hsa-mir-6774; hsa-mir-1538; hsa-mir-548i-1; hsa-mir-4440; hsa-miR-4423-5p; hsa-mir-196b; hsa-mir-8083; hsa-miR-3692-3p; hsa-miR-1234-3p; hsa-mir-4254; hsa-mir-4297; hsa-miR-1199-3p; hsa-mir-519d; hsa-mir-6794; hsa-mir-422a; hsa-miR-33a-3p; hsa-mir-548d-1; hsa-miR-6868-3p; hsa-mir-4669; hsa-mir-498; hsa-mir-4722; hsa-mir-4800; hsa-mir-548av; hsa-miR-6720-5p; hsa-mir-7855; hsa-mir-6809; hsa-miR-6771-3p; hsa-miR-3152-5p; hsa-mir-8075; hsa-mir-4776-2; hsa-mir-671; hsa-miR-4276; hsa-miR-4288; hsa-miR-1285-5p; hsa-mir-219a-1; hsa-miR-6835-3p; hsa-mir-4664; hsa-mir-138-2; hsa-miR-3614-5p; hsa-mir-593; hsa-mir-8089; hsa-mir-3944; hsa-mir-4739; hsa-miR-4646-3p; hsa-miR-23b-3p; hsa-mir-6127; hsa-miR-518d-3p; hsa-miR-5704; hsa-miR-103b; hsa-mir-892a; hsa-mir-541; hsa-mir-589; hsa-mir-7112-1; hsa-mir-7112-2; hsa-mir-3198-1; hsa-mir-3198-2; hsa-miR-7155-3p; hsa-mir-6772; hsa-miR-892a;

		hsa-miR-593-5p; hsa-mir-4675; hsa-miR-4522; hsa-mir-6075; hsa-miR-3145-5p; hsa-miR-4717-5p; hsa-miR-659-5p; hsa-mir-744; hsa-mir-6715b; hsa-miR-584-3p; hsa-miR-4794; hsa-mir-449b; hsa-miR-598-3p; hsa-mir-650; hsa-mir-518e; hsa-miR-4753-3p; hsa-miR-6861-3p; hsa-miR-1288-5p; hsa-mir-103b-1; hsa-mir-4635; hsa-mir-6753; hsa-mir-551a; hsa-mir-129-1; hsa-mir-129-2; hsa-mir-6810; hsa-miR-5685; hsa-mir-3155a;hsa-mir-4727; hsa-miR-1304-3p; hsa-mir-1299; hsa-mir-877; hsa-mir-4638; hsa-miR-7107-3p; hsa-mir-6816; hsa-miR-4661-5p; hsa-mir-4633; hsa-mir-1307; hsa-mir-6730; hsa-miR-6889-3p; hsa-mir-3189; hsa-miR-3183; hsa-mir-147a; hsa-miR-617; hsa-miR-216b-3p; hsa-miR-490-3p; hsa-miR-6822-3p; hsa-mir-662; hsa-mir-4432; hsa-mir-5001; hsa-let-7a-3p; hsa-miR-3682-5p; hsa-mir-5008; hsa-miR-488-5p; hsa-mir-548v; hsa-miR-605-5p; hsa-mir-3160-2; hsa-mir-2116; hsa-mir-4772; hsa-miR-2681-5p; hsa-miR-6857-3p; hsa-mir-4718; hsa-miR-31-3p; hsa-miR-602; hsa-mir-5589; hsa-miR-4469; hsa-mir-548i-3; hsa-mir-2861; hsa-mir-4489; hsa-mir-583; hsa-mir-1910; hsa-miR-382-3p; hsa-mir-3939; hsa-miR-4632-3p; hsa-mir-891a; hsa-miR-613; hsa-mir-4293; hsa-mir-6715a; hsa-mir-5572; hsa-miR-323b-3p; hsa-mir-4695; hsa-miR-6826-3p; hsa-miR-676-5p; hsa-mir-6881; hsa-mir-6507; hsa-mir-7702; hsa-miR-4659a-5p; hsa-miR-3664-5p; hsa-mir-5191; hsa-miR-6759-3p; hsa-mir-6802; hsa-mir-133b; hsa-miR-4263; hsa-mir-1273h; hsa-miR-3928-5p; hsa-miR-5189-3p; hsa-miR-548d-3p; hsa-miR-1269b; hsa-mir-3194; hsa-mir-7974; hsa-mir-6726; hsa-miR-1233-3p; hsa-mir-1293; hsa-miR-4325; hsa-miR-642a-5p; hsa-mir-3177; hsa-miR-661; hsa-miR-3150b-5p; hsa-mir-6889; hsa-miR-6855-3p; hsa-mir-6803; hsa-miR-383-3p; hsa-mir-4780; hsa-mir-1251; hsa-mir-4771-1; hsa-mir-4771-2; hsa-mir-7847; hsa-miR-7153-3p; hsa-miR-670-3p; hsa-miR-5187-3p; hsa-miR-5699-3p; hsa-mir-924; hsa-miR-6820-3p; hsa-mir-5687; hsa-miR-1207-3p; hsa-mir-604; hsa-mir-6088; hsa-mir-3683; hsa-miR-6799-3p; hsa-mir-3185; hsa-miR-516b-3p; hsa-miR-516a-3p; hsa-mir-6814; hsa-mir-4794; hsa-miR-4456; hsa-mir-216b; hsa-miR-3678-5p; hsa-mir-6830; hsa-miR-4778-3p; hsa-miR-490-5p; hsa-miR-1324; hsa-mir-659; hsa-miR-891b; hsa-mir-3200; hsa-miR-106a-3p; hsa-mir-486; hsa-mir-8064; hsa-mir-6766; hsa-mir-3180-4; hsa-mir-3180-5; hsa-miR-521; hsa-mir-3190; hsa-miR-7976; hsa-mir-4721; hsa-mir-5682; hsa-mir-617; hsa-mir-4315-1; hsa-mir-4315-2; hsa-mir-501; hsa-miR-3074-5p; hsa-miR-1914-5p; hsa-mir-7706; hsa-mir-6727; hsa-miR-494-5p; hsa-mir-585; hsa-mir-138-1; hsa-miR-1251-5p; hsa-miR-4653-5p; hsa-miR-616-5p; hsa-mir-371a; hsa-mir-5093; hsa-miR-1204; hsa-mir-5692a-1; hsa-mir-5692a-2; hsa-mir-7850; hsa-mir-4540; hsa-mir-6808; hsa-mir-346; hsa-mir-6849; hsa-mir-488; hsa-miR-4315; hsa-mir-4682; hsa-miR-182-3p; hsa-mir-6744; hsa-mir-548y; hsa-mir-938; hsa-miR-4642; hsa-miR-4709-5p; hsa-miR-5579-5p; hsa-miR-3972; hsa-miR-4307; hsa-mir-4725; hsa-mir-6834; hsa-mir-4484; hsa-miR-4740-5p; hsa-mir-4258; hsa-mir-1273f; hsa-mir-6781; hsa-miR-2117; hsa-mir-6824; hsa-mir-548i-2; hsa-miR-26a-1-3p; hsa-miR-3938; hsa-mir-4446; hsa-mir-4463; hsa-mir-892b; hsa-mir-1200; hsa-mir-1269b; hsa-miR-219a-5p; hsa-miR-497-3p; hsa-miR-3670; hsa-mir-4525; hsa-mir-4677; hsa-mir-548ay; hsa-mir-4732; hsa-miR-4731-3p; hsa-mir-1537; hsa-miR-15a-3p; hsa-miR-4524b-5p; hsa-mir-6783; hsa-mir-448; hsa-miR-3136-3p; hsa-mir-6811; hsa-mir-4744; hsa-mir-4493; hsa-mir-1276; hsa-miR-3941; hsa-mir-4328; hsa-miR-3189-5p; hsa-mir-5003; hsa-mir-6887; hsa-mir-578; hsa-mir-5685; hsa-mir-1287; hsa-mir-6807; hsa-mir-595; hsa-mir-431; hsa-mir-4642; hsa-miR-5010-3p; hsa-miR-6853-3p; hsa-mir-4536-1; hsa-miR-7114-3p; hsa-mir-4661; hsa-mir-8058; hsa-miR-6715b-3p; hsa-mir-153-2; hsa-mir-4756; hsa-miR-6859-3p; hsa-miR-2114-3p; hsa-miR-2355-5p; hsa-miR-222-5p; hsa-mir-6806; hsa-mir-8088; hsa-miR-517-5p; hsa-miR-621; hsa-miR-6764-3p; hsa-mir-24-1; hsa-miR-523-3p; hsa-mir-197; hsa-miR-6719-3p; hsa-mir-103b-2; hsa-mir-492; hsa-miR-4524a-5p; hsa-mir-4527; hsa-mir-6134; hsa-miR-4661-3p; hsa-mir-23a; hsa-mir-4663; hsa-miR-3157-3p; hsa-miR-6071; hsa-miR-548at-3p; hsa-mir-4467; hsa-miR-6769a-3p; hsa-mir-1178; hsa-miR-4796-5p; hsa-miR-1257; hsa-miR-519d-5p;hsa-miR-3684; hsa-mir-5681a; hsa-mir-5579; hsa-mir-3118-1; hsa-mir-3118-3; hsa-mir-8066; hsa-miR-4746-5p; hsa-miR-487b-5p; hsa-mir-1184-1; hsa-mir-1184-2; hsa-mir-1184-3; hsa-mir-6716; hsa-mir-6770-1; hsa-mir-6770-2; hsa-mir-6770-3; hsa-mir-1539; hsa-mir-140; hsa-miR-1298-5p; hsa-miR-454-5p; hsa-mir-150; hsa-miR-708-3p; hsa-mir-4268; hsa-mir-3940; hsa-mir-3130-2; hsa-mir-4762; hsa-mir-8056; hsa-mir-5190; hsa-miR-4493; hsa-miR-3614-3p; hsa-mir-3670-1; hsa-mir-3670-2; hsa-miR-7853-5p; hsa-mir-4737; hsa-mir-6512; hsa-let-7d; hsa-mir-374c; hsa-miR-645; hsa-mir-4269; hsa-mir-8077; hsa-miR-3925-3p; hsa-mir-6513; hsa-mir-5002; hsa-miR-4659b-3p; hsa-mir-5186; hsa-miR-101-5p; hsa-mir-7153; hsa-miR-5003-5p; hsa-miR-96-3p; hsa-mir-2114; hsa-mir-1-1; hsa-mir-504; hsa-miR-6876-3p; hsa-mir-181a-1; hsa-mir-378a; hsa-miR-2682-5p; hsa-mir-548h-1; hsa-miR-548ao-3p; hsa-miR-4637; hsa-mir-3663; hsa-mir-5706; hsa-miR-141-5p; hsa-miR-215-3p; hsa-mir-7151; hsa-mir-451b; hsa-mir-4509-1; hsa-mir-4509-2; hsa-mir-4509-3; hsa-mir-3664; hsa-mir-4535; hsa-mir-4456; hsa-mir-4804; hsa-miR-3140-5p; hsa-miR-301a-5p; hsa-mir-1249; hsa-mir-3183; hsa-mir-3151; hsa-mir-7848; hsa-miR-548au-3p; hsa-mir-2110; hsa-mir-4292; hsa-miR-203b-5p; hsa-mir-106a; hsa-miR-5586-5p; hsa-mir-4799; hsa-miR-3913-3p; hsa-mir-3169; hsa-mir-331; hsa-mir-4421; hsa-let-7c; hsa-mir-888; hsa-mir-4447; hsa-mir-5696; hsa-mir-1271; hsa-mir-375; hsa-mir-191; hsa-mir-380; hsa-mir-623; hsa-mir-3118-2; hsa-mir-203b; hsa-miR-495-5p; hsa-mir-3120; hsa-mir-7-1; hsa-mir-7155; hsa-miR-3924; hsa-miR-302b-3p; hsa-mir-383; hsa-miR-4308; hsa-mir-433; hsa-miR-3117-5p; hsa-mir-518d; hsa-miR-1271-3p; hsa-mir-7973-1; hsa-mir-519c; hsa-mir-5582; hsa-mir-558; hsa-miR-4781-3p; hsa-mir-6882; hsa-mir-3678; hsa-miR-6755-5p; hsa-mir-412; hsa-miR-4693-5p; hsa-mir-5702; hsa-mir-499a; hsa-miR-4797-3p; hsa-mir-5087; hsa-mir-521-2; hsa-mir-490; hsa-miR-3133; hsa-mir-4659b; hsa-miR-146a-3p; hsa-mir-4477b; hsa-miR-4735-5p; hsa-mir-218-1; hsa-miR-372-5p; hsa-mir-3972; hsa-mir-199a-1; hsa-miR-6839-3p; hsa-miR-562; hsa-miR-5004-3p; hsa-mir-4318; hsa-mir-3118-5; hsa-mir-548h-3; hsa-miR-4272; hsa-mir-4764; hsa-mir-3165; hsa-mir-4789; hsa-miR-649; hsa-miR-1197; hsa-mir-23b; hsa-mir-1302-4

GSE18509	**LFC > 0**	hsa-miR-421; hsa-miR-615; hsa-miR-454-3p; hsa-miR-7; hsa-miR-766; hsa-miR-196a; hsa-miR-146b; hsa-miR-132; hsa-miR-93; hsa-miR-425-5p; hsa-miR-185; hsa-miR-9; hsa-miR-130b; hsa-miR-142-5p; hsa-miR-22; hsa-miR-9*; hsa-miR-106b; hsa-miR-18a; hsa-miR-142-3p; hsa-miR-155; hsa-miR-20b; hsa-miR-21.
	LFC < 0	hsa-miR-205; hsa-miR-200c; hsa-miR-211; hsa-miR-203; hsa-miR-204; hsa-miR-23b; hsa-miR-200b; hsa-miR-149; hsa-miR-141; hsa-miR-125b; hsa-miR-193b; hsa-miR-27b; hsa-let-7b; hsa-miR-455; hsa-miR-768-3p; hsa-miR-200a; hsa-miR-214; hsa-miR-125a; hsa-miR-224; hsa-miR-564; hsa-miR-197; hsa-miR-429
GSE54492	**LFC > 0**	hsa-miR-193b; hsa-miR-609; hsa-miR-769-3p; hsa-miR-647
	LFC < 0	hsa-miR-126*; hsa-miR-211; hsa-miR-661; hsa-miR-29c; hsa-miR-923; hsa-miR-644; hsa-miR-509-5p; hsa-miR-149*; hsa-miR-30e*; hsa-miR-30e; hsa-miR-513-3p; hsa-miR-151-3p; hsa-miR-509-3p; hsa-miR-638; hsa-miR-596; hsa-miR-378; hsa-miR-659; hsa-miR-98; hsa-miR-17*; hsa-miR-30d; hsa-miR-708*; hsa-miR-30a; hsa-miR-30a*; hsa-miR-760; hsa-miR-30d*; hsa-miR-188-5p; hsa-miR-106b*; hsa-miR-135a*; hsa-miR-335*; hsa-miR-124; hsa-miR-432*; hsa-miR-584; hsa-let-7f; hsa-miR-26b; hsa-miR-130a; hsa-miR-296-5p; hsa-miR-454; hsa-miR-34a*; hsa-miR-650; hsa-miR-941; hsa-miR-195; hsa-miR-215; hsa-miR-643; hsa-miR-628-5p; hsa-miR-146a; hsa-miR-885-3p; hsa-miR-181a*; hsa-miR-610; hsa-miR-361-3p; hsa-miR-422a; hsa-miR-29b; hsa-miR-148b*; hsa-miR-184; hsa-miR-20a*; hsa-miR-487a; hsa-miR-590-5p; hsa-miR-18a*; hsa-miR-328; hsa-miR-564; hsa-miR-489; hsa-miR-186; hsa-miR-15b*; hsa-miR-892b; hsa-miR-16-1*; hsa-miR-340*; hsa-miR-571; hsa-miR-20a; hsa-let-7a; hsa-miR-628-3p; hsa-miR-190b; hsa-miR-202; hsa-miR-101; hsa-miR-502-5p; hsa-miR-19a; hsa-miR-374; hsa-miR-148a; hsa-miR-29a*; hsa-miR-519d; hsa-miR-33a*; hsa-miR-181c; hsa-miR-10a*; hsa-miR-26a; hsa-miR-519e; hsa-miR-565; hsa-miR-29c*; hsa-miR-586; hsa-let-7i*; hsa-miR-483-5p; hsa-miR-942; hsa-miR-142-3p; hsa-miR-516-3p; hsa-miR-672; hsa-miR-545; hsa-let-7e; hsa-miR-181a-2*; hsa-miR-768-3p; hsa-miR-26a-1*; hsa-miR-766; hsa-miR-618; hsa-miR-491-5p; hsa-miR-15a; hsa-miR-559; hsa-miR-580; hsa-miR-19b-1*; hsa-miR-106a; hsa-miR-629*; hsa-miR-93*; hsa-miR-625*; hsa-miR-601
GSE34460	**LFC > 0**	hsa-miR-720; hsa-miR-3663-3p; hsa-miR-21; hsa-miR-142-3p; hsa-miR-155; hsa-miR-21*
	LFC < 0	hsa-miR-429; hsa-miR-204; hsa-miR-200a; hsa-miR-455-5p; hsa-miR-664; hsa-miR-183; hsa-miR-224*; hsa-let-7a; hsa-miR-26a

Meta-analysis

The number of significantly differentially-expressed miRNAs by meta-analysis (MA) and the studies included in each scenario is shown in Figure 2 and Table S2. In the ulceration approach, 271 miRNA were found to be up-regulated in the ulcerated tissue, versus the 1327 miRNA analyzed. In the diagnosis scenario, only 14 miRNAs were up-regulated, two more than when the metastatic tissue is not taken into account. Detailed miRNA differentially-expressed by meta-analysis can be observed in Table 3.

Table 3. Results of meta-analyses by comparison. Description of significant miRNAs by comparisons: PMBN (Primary melanoma vs Benign nevus), PMMMBN (Primary melanoma and Metastatic melanoma vs Benign nevus), UMNU (Ulcerated melanoma vs non-ulcerated melanoma), IDF (impact of the diagnosis of the disease in females). Significant miRNAs are grouped according to the sign of their Log fold-change (LFC).

Contrast	LFC	Significant miRNAs
UM vs NUM	**LFC > 0**	hsa-miR-1205, hsa-miR-1254, hsa-miR-1289, hsa-miR-1307, hsa-miR-375, sa-miR-519c-3p, hsa-miR-548d-5p, hsa-miR-556-3p, hsa-miR-568, hsa-miR-590-3p, hsa-miR-604, hsa-miR-606, hsa-miR-633, hsa-miR-649, hsa-miR-1, hsa-miR-101*, hsa-miR-10b*, hsa-miR-1180, hsa-miR-1181, hsa-miR-1206, hsa-miR-1208, hsa-miR-1225-3p, hsa-miR-1231, hsa-miR-1245, hsa-miR-1263, hsa-miR-1266, hsa-miR-1267, hsa-miR-1269, hsa-miR-1277, hsa-miR-1282, hsa-miR-1288, hsa-miR-129-3p, hsa-miR-1295, hsa-miR-1305, hsa-miR-130a*, hsa-miR-132, hsa-miR-133a, hsa-miR-134, hsa-miR-143*, hsa-miR-144*, hsa-miR-147, hsa-miR-148a*, hsa-miR-149, hsa-miR-150*,hsa-miR-153, hsa-miR-1537, hsa-miR-15a*, hsa-miR-15b*, hsa-miR-16-1*, hsa-miR-181c, hsa-miR-181c*, hsa-miR-182*, hsa-miR-18a*, hsa-miR-192, hsa-miR-192*, hsa-miR-200c*, hsa-miR-216a, hsa-miR-224, hsa-miR-2276, hsa-miR-27b*, hsa-miR-28-3p, hsa-miR-301b, hsa-miR-302b, hsa-miR-302c, hsa-miR-302d*, hsa-miR-329, hsa-miR-33b, hsa-miR-33b*, hsa-miR-345, hsa-miR-34a*, hsa-miR-367, hsa-miR-371-3p, hsa-miR-372, hsa-miR-373, hsa-miR-374a*, hsa-miR-378*, hsa-miR-380, hsa-miR-380*, hsa-miR-384, hsa-miR-409-3p, hsa-miR-410, hsa-miR-411*, hsa-miR-432, hsa-miR-449b, hsa-miR-450b-5p, hsa-miR-495, hsa-miR-499-5p, hsa-miR-503, hsa-miR-504, hsa-miR-511, hsa-miR-516a-3p, hsa-miR-516b*, hsa-miR-517*, hsa-miR-517a, hsa-miR-517b, hsa-miR-518c, hsa-miR-518e, hsa-miR-518f, hsa-miR-519a, hsa-miR-520e, hsa-miR-524-5p, hsa-miR-526b, hsa-miR-539, hsa-miR-545, hsa-miR-545*, hsa-miR-548j, hsa-miR-548l, hsa-miR-561, hsa-miR-569, hsa-miR-577, hsa-miR-580, hsa-miR-586, hsa-miR-616, hsa-miR-619, hsa-miR-621, hsa-miR-624, hsa-miR-624*, hsa-miR-651, hsa-miR-658, hsa-miR-7-1*, hsa-miR-758, hsa-miR-759, hsa-miR-875-3p, hsa-miR-875-5p, hsa-miR-888, hsa-miR-92a-2*, hsa-miR-941, hsa-miR-96*, hsa-miR-362-5p, hsa-miR-105*, hsa-miR-1185, hsa-miR-1207-5p, hsa-miR-1233, hsa-miR-1250, hsa-miR-1256, hsa-miR-1268, hsa-miR-127-3p, hsa-miR-1322, hsa-miR-146a*, hsa-miR-19a*, hsa-miR-19b-2*, hsa-miR-216b, hsa-miR-219-1-3p, hsa-miR-222*, hsa-miR-26a-1*, hsa-miR-30c-2*, hsa-miR-324-5p, hsa-miR-34c-3p, hsa-miR-376c, hsa-miR-431*, hsa-miR-432*, hsa-miR-488*, hsa-miR-493, hsa-miR-493*, hsa-miR-497*, hsa-miR-520g, hsa-miR-541*, hsa-miR-548f, hsa-miR-548o, hsa-miR-548p, hsa-miR-597, hsa-miR-598, hsa-miR-628-5p, hsa-miR-656, hsa-miR-671-3p, hsa-miR-708*, hsa-miR-744*, hsa-miR-769-5p, hsa-miR-770-5p, hsa-miR-889, hsa-miR-924, hsa-miR-100*, hsa-miR-105, hsa-miR-1182, hsa-miR-1234, hsa-miR-1251, hsa-miR-1292, hsa-miR-1296, hsa-miR-136*, hsa-miR-138-2*, hsa-miR-154, hsa-miR-19b-1*, hsa-miR-218, hsa-miR-23b*, hsa-miR-29c*, hsa-miR-302b*, hsa-miR-302d, hsa-miR-323-5p, hsa-miR-335*, hsa-miR-363*, hsa-miR-379, hsa-miR-452*, hsa-miR-484, hsa-miR-520f, hsa-miR-524-3p, hsa-miR-548h, hsa-miR-559, hsa-miR-570, hsa-miR-579, hsa-miR-627, hsa-miR-644, hsa-miR-646, hsa-miR-944, hsa-miR-95, hsa-miR-1178, hsa-miR-1179, hsa-miR-1238, hsa-miR-20a*, hsa-miR-218-1*, hsa-miR-526b*, hsa-miR-543, hsa-miR-653, hsa-miR-662, hsa-miR-1271, hsa-miR-219-5p, hsa-miR-302a*, hsa-miR-654-3p, hsa-miR-615-5p, hsa-miR-30a*, hsa-miR-555, hsa-miR-618, hsa-miR-1226*, hsa-miR-139-5p, hsa-miR-16-2*, hsa-miR-541, hsa-miR-588, hsa-miR-502-5p, hsa-miR-1324, hsa-miR-194, hsa-miR-611, hsa-miR-182, hsa-miR-18b, hsa-miR-183, hsa-miR-107, hsa-miR-340, hsa-miR-151-3p, hsa-miR-492, hsa-miR-99b, hsa-miR-373*, hsa-miR-10a, hsa-miR-103, hsa-miR-130b, hsa-miR-185, hsa-miR-191, hsa-miR-138-1*, hsa-miR-423-3p, hsa-miR-20b, hsa-miR-140-3p, hsa-miR-15a, hsa-miR-20a, hsa-miR-720, hsa-miR-331-3p, hsa-miR-874, hsa-miR-30a, hsa-miR-1301, hsa-miR-15b, hsa-miR-629*, hsa-miR-146a, hsa-miR-22, hsa-miR-17, hsa-miR-106a, hsa-miR-106b, hsa-miR-1274a, hsa-miR-16, hsa-miR-29a, hsa-miR-93, hsa-miR-21, hsa-miR-363-3p, hsa-miR-196b-5p, hsa-miR-135b-5p, hsa-miR-451, hsa-miR-223-3p
	LFC < 0	hsa-miR-376c-5p, hsa-miR-518e-3p, hsa-miR-489-3p, hsa-miR-624-3p, hsa-miR-487b-5p,hsa-miR-133a-5p, hsa-miR-517c-3p+hsa-miR-519a-3p, hsa-miR-758-3p+hsa-miR-411-3p, hsa-miR-552-3p, hsa-miR-499a-5p, hsa-miR-580-3p, hsa-miR-5010-5p, hsa-miR-205, hsa-miR-381-5p, hsa-miR-450a-2-3p, hsa-miR-203, hsa-miR-302a-3p, hsa-miR-6720-3p, hsa-miR-523-3p, hsa-miR-548g-3p, hsa-miR-301a-5p, hsa-miR-134-3p, hsa-miR-210-5p, hsa-miR-1245b-5p, hsa-miR-6721-5p, hsa-miR-548b-5p, hsa-miR-208b-3p, hsa-miR-585-3p,

		hsa-miR-219a-5p, hsa-miR-200c, hsa-miR-526a+hsa-miR-518c-5p+hsa-miR-518d-5p, hsa-miR-584-3p, hsa-miR-376b-3p, hsa-miR-29b-1*, hsa-miR-200b, hsa-miR-3196, hsa-miR-299-5p, hsa-miR-296-3p, hsa-miR-382, hsa-miR-876-3p, hsa-miR-338-5p, hsa-miR-378b, hsa-miR-601, hsa-miR-214, hsa-miR-877*, hsa-miR-1976, hsa-miR-1272, hsa-miR-1183
PM+MM vs BN	**LFC > 0**	hsa-miR-766, hsa-miR-584, hsa-miR-142-5p, hsa-miR-22, hsa-miR-3132, hsa-miR-3195, hsa-miR-3138, hsa-miR-3610, hsa-miR-142-3p, hsa-miR-3911, hsa-miR-21, hsa-miR-3156-5p, hsa-miR-3648, hsa-miR-1973
	LFC < 0	hsa-miR-125b, hsa-miR-23b, hsa-miR-100, hsa-miR-101, hsa-miR-26a, hsa-miR-509-3p, hsa-miR-508-3p, hsa-let-7a, hsa-miR-26b, hsa-let-7c, hsa-miR-378, hsa-let-7d, hsa-miR-23a, hsa-let-7f, hsa-miR-30c, hsa-miR-365, hsa-miR-361-5p
PM vs nevus	**LFC > 0**	hsa-miR-584, hsa-miR-22, hsa-miR-142-5p, hsa-miR-3132, hsa-miR-3195, hsa-miR-3138, hsa-miR-3610, hsa-miR-21, hsa-miR-3911, hsa-miR-3156-5p, hsa-miR-3648, hsa-miR-1973
	LFC < 0	hsa-miR-211, hsa-miR-101, hsa-miR-100, hsa-miR-205, hsa-miR-203, hsa-miR-23b, hsa-miR-509-3p, hsa-miR-508-3p, hsa-miR-193b, hsa-let-7c, hsa-let-7d, hsa-miR-30c, hsa-miR-23a, hsa-miR-365, hsa-miR-195, hsa-miR-361-5p

DISCUSSION

Melanoma is the only tumor in which a lesion of just over 1 millimeter can pose a high risk of metastasis. Studies of the disease have identified certain phenotypic traits as risk factors for its development, such as fair skin, a high number of nevi, being a transplant patient, or being prone to sunburns (CNIO, 2022).

However, at a molecular level, although various genetic and epigenetic alterations have been identified, as well as immune evasion processes, no molecular marker has been approved as an indicator of the disease's prognosis (CNIO, 2022). Therefore, in conjunction with the evident need for early disease detection, the primary objective of this work is to detect and understand the various differential molecular mechanisms behind melanoma.

To achieve this, a systematic review of studies was conducted following the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Page et al., 2021). The systematic review aims to gather evidence that meets predefined eligibility criteria to answer a specific research question, in this case, our objective. This method also helps minimize bias by using explicit and systematically documented methods established in advance with a protocol (Higgins and Thomas, 2021). After the systematic review, an exploratory data analysis was

conducted, allowing for the identification of sample characteristics and methodological specifics of each of the studies. The meta-analysis was employed as the method to integrate the data from the studies included in each of the outlined scenarios. This widely-used method increased the statistical power by increasing the sample size and consequently improved the precision in identification, thereby achieving the goals set in this work (Higgins and Thomas, 2021; Normand, 1999).

On another note, it is worth mentioning that in the course of the disease's development, some studies have established differences between sexes, which are more likely due to biological rather than behavioral differences, such as the role of sex steroid hormones in immunomodulation (Schwartz et al., 2019). However, women continue to be underrepresented in biomedical research, and despite these differences being crucial for identifying biomarkers, at best, the variable of sex is only included as a patient's data point and not with the aim of finding differences between men and women (Kim et al., 2010). Consequently, in our selection of studies, we have found very few that incorporated this variable into their data. Therefore, when classifying the samples in the exploratory analysis, we could not consider it.

However, due to the diversity in the nature of tissues found among the studies that ultimately became candidates for selection, it was decided to discard those that did not fit into any of the outlined scenarios. This decision was made because the goal of the diagnostic scenario was to discover differences in miRNA expression between healthy tissue and tissue with melanoma, with the aim of establishing future markers for early disease diagnosis.

Concerning the goal of the progression scenario, we delineate two distinct contrasts. Firstly, we aimed to assess survival, but due to the marked heterogeneity in patient characteristics and intrinsic disease attributes, we encountered significant challenges in pinpointing statistically significant biomarkers. Secondly, we honed our focus on forecasting disease progression,

specifically ulceration. Within this context, we successfully identified biomarkers that enhance our comprehension of the disease dynamics and patient outcomes.

This study has established the efficacy of meta-analysis as a robust tool for aggregating and appraising data derived from diverse investigations within a specific domain. In our case, this approach was applied to comprehensively review studies focusing on miRNAs in the context of melanoma.

Furthermore, the methodology employed in this investigation facilitated the discernment of distinct miRNA expression patterns in two key disease detection scenarios, encompassing both metastatic and primary melanomas. Additionally, this analytical framework led to the identification of pertinent biomarkers associated with ulceration. It is worth noting that this study underscores the imperative of enhancing awareness among the scientific community regarding the inclusion of sex as a variable in melanoma-related biomedical research, along with the urgency for standardizing data recording procedures in clinical trials.

In aggregate, the findings from this study provide a solid foundation for subsequent research endeavors aimed at undertaking a functional characterization of the significant miRNAs discovered. This pursuit offers a novel and indispensable perspective, particularly in the context of personalized medicine and the precision-oriented landscape of biomedical investigations within the domain of melanoma research.

MATERIALS & METHODS

Workflow

The workflow for each of the selected studies was as follows: i) Systematic review; ii) Data acquisition and preprocessing; iii) exploratory analysis of data; iv) Data normalization; v) Contrast groups and scenarios setting up and DE analysis; vi) Meta-analysis; vii) Functional enrichment; and viii) Biological and clinical interpretation

Studies were grouped for meta-analysis based on tissue similarity and scenario of contrast.

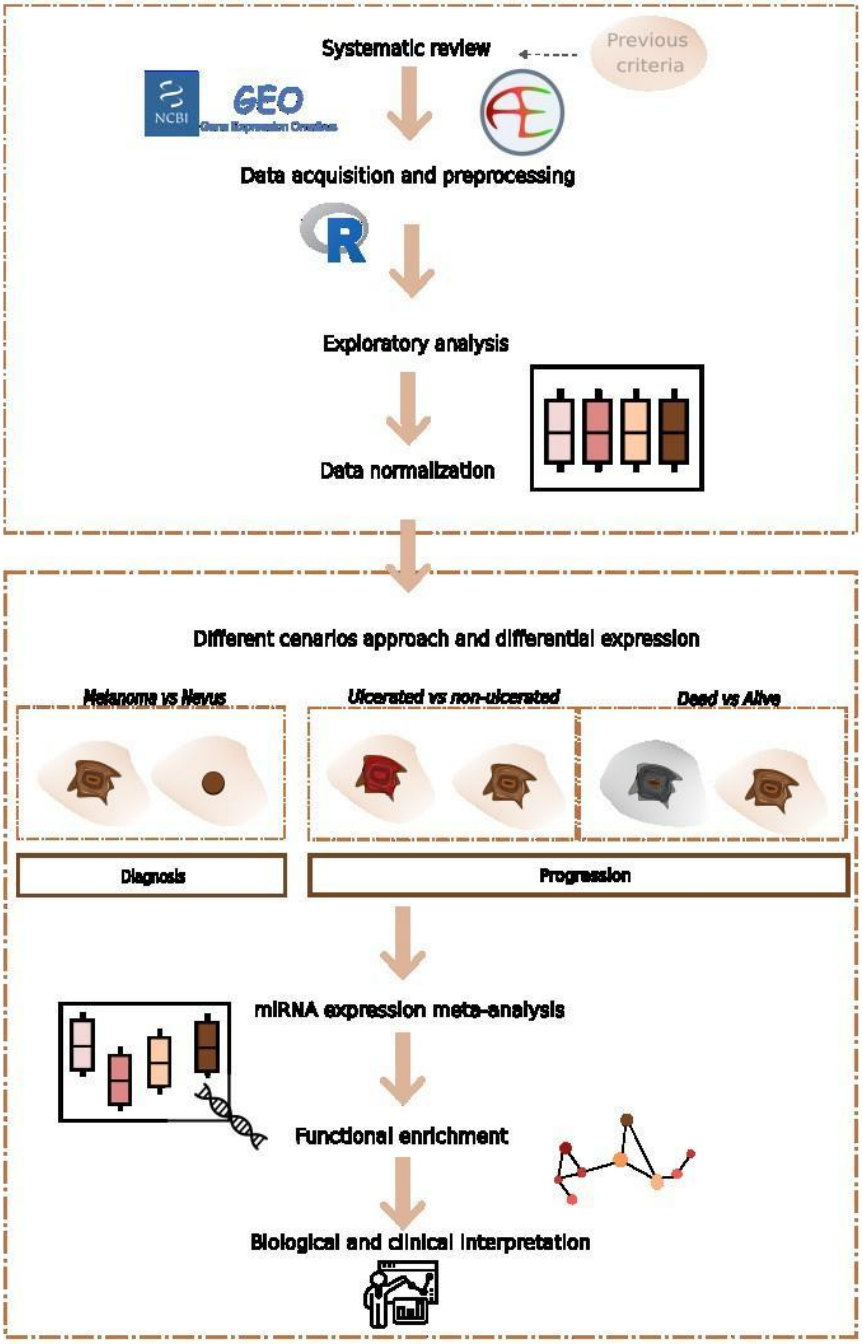

Figure 3. Bioinformatics strategy pipeline.

Systematic Review

A systematic review is the process by which empirical evidence is examined to determine if a methodology meets certain eligibility criteria for addressing specific questions. Its methodology

involves setting clear objectives, conducting a systematic search for studies that meet the established eligibility criteria, assessing their validity, and synthesizing the obtained results (Liberati et al., 2009).

In our case, the systematic review involved conducting an exhaustive search for studies with microarray data of miRNAs in the *Gene Expression Omnibus (GEO*) and *ArrayExpress* repositories. The search filters included the following keywords: melanoma, microRNAs, microRNA, *Homo sapiens*, and expression profiling by array. Additionally, a filter was applied for studies with a sample size greater than 12.

In the studies obtained through the search, filtering was carried out based on the following criteria: i)Focused on melanoma; ii)Studies analyzing miRNAs; iii)Tissue samples from Homo sapiens (excluding cell lines); iv) Minimum sample size (n minimum = 12); v)Samples without treatment.

Furthermore, studies with a sample size of less than 12, those not exclusively focused on melanoma, those centered on uveal melanoma, and those containing cell lines or samples that had received any form of treatment were excluded.

The analysis and presentation of results were conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al., 2021).

Data acquisition and data preprocessing

Once the selected studies were obtained, the normalized data for each of them were downloaded. Subsequently, the studies were individually analyzed to combine the results later. Additionally, during the individual analysis, filtering was performed to retain only Homo sapiens-specific miRNAs, and probes used as controls in the original analysis were removed.

After the individual exploratory analysis, data from studies that were not normalized were standardized, and samples exhibiting anomalous behavior were excluded. This approach started with all selected studies having normalized data to avoid introducing new biases and, therefore, greater variability in subsequent analyses.

All data processing was carried out using the R programming language (R Core Team, 2021) from the RStudio graphical interface. The packages used for data download, processing, and analysis included the following: limma (Ritchie et al., 2015); ggplot2 (Wickham, 2009); dplyr (Wickham, 2021a); Biobase (Huber et al., 2015); factoextra (Kassambara and Mundt, 2020); impute (Hastie and Tibshirani, 2021), and stringr (Wickham, 2021b).

Exploratory analysis

Before conducting any type of analysis, it is crucial to perform an individual exploratory examination of each of the selected studies. The purpose is to take into account the methodology used in each of them and facilitate the correct interpretation of the results. This process also aims to generate and standardize the various results for subsequent combination in the meta-analysis. Furthermore, the exploratory analysis also allowed for the observation of whether different samples followed any patterns related to the variables of interest.

In addition, a descriptive statistical analysis was conducted to help identify anomalous data and behaviors in the data, as well as to examine the distribution of different groups of interest.

The descriptive analysis consisted of: i). Box plots for the identification of the presence or absence of anomalies in the data. If anomalies were found, they were corrected. ii) Sample clustering to observe potential groupings within the data. This analysis also facilitated the selection of different contrast groups. iii) Principal Component Analysis (PCA), for the observation of outliers in the data and potential relationships between data groupings.

Different Scenarios Approach

Following the examination of sample types, two distinct methodological approaches were introduced: one oriented towards diagnostic considerations and the other focusing on the assessment of disease progression.

In the diagnostic context, the research involved the comparative analysis of primary cutaneous melanoma and/or cutaneous metastatic melanoma specimens against nevi specimens. Conversely, in the progression framework, two specific contrasts were explored. The first involved the comparison of ulcerated and non-ulcerated primary cutaneous melanomas, while the second pertained to the comparative analysis of melanoma tissues from patients who had succumbed to the disease with those who had successfully survived melanoma (survival approach).

A number of studies were found to be applicable to different contrasts, necessitating the tailored selection of samples for each study in alignment with the particular research objectives.

Differential Expression Analysis

A differential expression analysis was conducted for each of the studies. Comparisons were made between the different contrast groups defined in each study. The main objective of this analysis was to identify differentially expressed miRNAs to potentially reveal differences in the proposed contrasts.

Meta-analyses

Meta-analysis is a type of scientific research that aims to objectively and systematically integrate the results of empirical studies on a specific subject, making it a valuable tool in the field of medical sciences.

The technique of meta-analysis involves reviewing and combining the results of differential expression from various studies in the same field of study. By increasing the available sample size, the results become more statistically robust (Liberati et al., 2009; Normand, 1999).

Furthermore, combining different results also allows for the removal of background noise caused by the platform used, experimental design, or even confers statistical significance to parameters that wouldn't have it if analyzed separately. It's a highly versatile technique that can be applied at various levels (transcript, differential expression, or functional) (García-García, 2016).

To conduct the meta-analysis and set the approach for the study, the following aspects were considered: i)study design; ii) inclusion/exclusion criteria for studies; iii) variables of interest to be evaluated; iv) literature search; v) study selection; vi) evaluation of study quality; vii) data extraction; viii) Statistical analysis.

Furthermore, R programming language (R Core Team, 2021) was used for conducting the meta-analysis, with the metafor package (Viechtbauer, 2010) being employed.

DECLARATIONS

Conflict of interest

All the authors declare no conflict of interest.

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Data availability

The data used for the analyses described in this work are publicly available at GEO [23] and ArrayExpress [24]. The accession numbers of the GEO datasets downloaded are GSE7621, GSE8397, GSE20146, GSE20295, and GSE28894. The accession numbers of the ArrayExpress datasets downloaded are E-MTAB-1194 and E-MEXP-1416.

Code availability

The code developed for the analyses described in this work has been made publicly available at GitHub (https://github.com/adlpecer/PD_GeneMetaanalysis). All software and versions used are detailed in Additional file 2: Table S2.

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TABLES

Table 1. Description of selected studies. The characteristics of the studies are shown as available in its respective database.

Table 2. Description of significant miRNAs by study. Significant miRNAs are grouped according to the sign of their Log fold-change (LFC).

Table 3. Results of meta-analyses by comparison. Description of significant miRNAs by comparisons: PMBN (Primary melanoma vs Benign nevus), PMMMBN (Primary melanoma and Metastatic melanoma vs Benign nevus), UMNU (Ulcerated melanoma vs non-ulcerated melanoma), IDF (impact of the diagnosis of the disease in females). Significant miRNAs are grouped according to the sign of their Log fold-change (LFC).

FIGURE LEGENDS

Figure 1. PRISMA diagram of the systematic review.

Figure 2. Number of significantly differentially-expressed miRNAs by study . Description of significant miRNAs by comparisons: Primary melanoma vs Benign nevus, Primary melanoma and metastatic melanoma vs Benign nevus, and ulcerated melanoma vs non-ulcerated melanoma). LFC: log-FoldChange.

Figure 3. Bioinformatics strategy pipeline

SUPPLEMENTARY MATERIAL

Table S1. Number of significantly differentially-expressed miRNAs by study . Description of significant miRNAs by comparisons: PMBN (Primary melanoma vs Benign nevus), PMMMBN (Primary melanoma and Metastatic melanoma vs Benign nevus), MMPM(metastatic melanoma vs primario) UMNU (Ulcerated melanoma vs non-ulcerated melanoma), and A vs DM (alive vs Dead by melanoma). Significant miRNAs are grouped according to the sign of their Log fold-change (LFC). TAM: Total Analyzed miRNAs

Accession	COMPARISON														
	PM vs BN			MM vs BN			MM vs PM			UM vs NU			DM vs A		
	LFC > 0	LFC < 0	TAM	LFC > 0	LFC < 0	TAM	LFC > 0	LFC < 0	TAM	LFC > 0	LFC < 0	TAM	LFC > 0	LFC < 0	TAM
E-MTAB-4915	648	898	4603	648	648	4603	-	-	-	-	-	-	-	-	-
GSE18509	-	-	-	22	22	470	-	-	-	-	-	-	-	-	-
GSE54492	-	-	-	-	-	-	4	109	661	-	-	-	-	-	-
GSE34460	0	15	1205	0	15	1205	-	-	-	-	-	-	-	-	-
GSE59334	-	-	-	-	-	-	-	-	-	-	-	-	0	0	
GSE62370	22	36	820	22	36	820	-	-	-	2	9	820	0	0	820
GSE62371	-	-	-	-	-	-	-	-	-	4	2	1193	0	0	1193
GSE211090	-	-	-	-	-	-	-	-	-	0	0	798	-	-	-
GSE211092	-	-	-	-	-	-	-	-	-	0	0	798	-	-	-

Table S2. Results of meta-analyses by comparison. Description of significant miRNAs by comparisons: PMBN (Primary melanoma vs Benign nevus), PMMMBN (Primary melanoma and Metastatic melanoma vs Benign nevus), UMNU (Ulcerated melanoma vs non-ulcerated melanoma), IDF (impact of the diagnosis of the disease in females). Significant miRNAs are grouped according to the sign of their Log fold-change (LFC).

Comparison	LFC > 0	LFC < 0	Total analyzed miRNA	Number of studies
UM vs NUM	271	48	1327	4
PM+MM vs BN	14	17	1153	4
PM vs nevus	12	16	1127	3